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SM Journal of Pediatrics

Research Article

Commentary: Environmental Medicine: The Role of Epigenetic Mechanisms

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Article Information

Received date: Aug 29, 2017
Accepted date: Sep 20, 2017
Published date: Sep 25, 2017

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Keywords Epigenetic; Human adult stem cells; Gap junctional intercellular communication; Barker hypothesis

Abstract

With the current emphasis on delivering accurate diagnosis, prognosis and treatments to prevent and treat human diseases, it is critical that one understands the mechanistic bases for the pathogenesis of both acute and chronic diseases. Although "Environmental Medicine" involves the whole spectrum of clinical disciplines from obstetrics, pediatrics to geriatrics and involves the potential roles of genetics in the onset of any diseases that to be associated with some environmental factor(s), the roles of "epigenetic" toxicological mechanisms seems to be largely ignored in "personalized medicine", or "precision medicine" where the use of genetic information has played a major role in certain aspects of personalized medicine. With the recent development of sophisticated molecular technologies, various current paradigms, some having origins in past insights to the origin of "genetic or heredity diseases" or in the discovery of environmental agents (radiation, toxic chemicals or pathogenic biological organisms), there has been some confusions of how these agents contribute to these diseases.

A short "Commentary" is proposed to assist in sorting out these various factors contributing to "genetic" and "environmental" causes of these diseases. In brief, it will be assumed that the singular concept that genes or environmental factors, alone, are not "the" cause of any disease, but that complex interactions are needed to affect disease pathogeneses. This will involve our current understanding that mutations (gene or chromosomal), cell death and alterations of gene expression at the transcriptional, translational and posttranslational levels (epigenetic change) are involved in the toxicological mechanisms of the pathogenesis of birth defects, cancer, cardiovascular diseases, inflammatory diseases, reproductive- and neurological-disorders. Much of our global disease burden is the result of a collision of glacial-speed biological evolution of genes needed for survival and reproduction with the laser speed cultural evolution. Lastly, with the recent discovery of organ-specific adult stem cells, alteration of the numbers (increase or decrease) of these organ-specific stem cells could provide the mechanistic basis for the risk of various stem-cell related diseases later in life (The Barker hypothesis).

"Since the mechanism of cell communication itself is universal in biology, in keeping with a Kuhnian paradigm shift. This approach may even elucidate the nature and evolution of consciousness as a manifestation of the cellular continuum from unicellular to multicellular life. We need such a functional genomic mechanism for the process of evolution if we are to make progress in biology and medicine [1]".

Introduction

While the early view that heredity must have had a role in some diseases even at a time when evil spirits, transgressions against one's god(s), and other non-scientific causes, with the discovery of DNA being the basis for heredity characteristics of our genes on our chromosomes, the possibility that alterations of the molecular nature of our genes or the numbers of our chromosomes (gene or chromosomal mutations, respectively) had been offered. With today's understanding of the role of our genomic DNA, there are specific genes and non-coding DNA associated with many diseases [2]. In addition, the traditional genes that code for specific proteins and functions, themselves, can be altered by "splice variants" [3] usually induced by environmental factors.

Here in lies the origin of a couple misleading views that gene mutations "caused" diseases. In the current field of cancer research, gene mutations are viewed as the "drivers" of cancer [4] and have opened up a global "cottage industry" in "precision medicine" [5].

In a more realistic view of DNA playing a role in our state of "normal" health or in a disease, we should view DNA, as did Rene Dubos when he stated:

"We resemble our progenitors because we derive from them our genetic endowment; but our genes do not determine traits by which we know a person. They only govern the responses that the person takes to the environmental [dietary] stimuli. Individuality progressively emerges from those responses" [6].

In other words, let us view our inherited DNA as the "blue print" of a home to be built. However, that home can only be actuated by carpenters, plumbers, electricians, painters, etc. which work from the blueprint. The actual house is then the result of an interaction of both the genetic material (genes and chromosomes) or the "blue print" and the specific environmental factors (physical, chemical, microbiological, nutritional/dietary, behavioral, psychological, social, cultural) or the carpenters,

plumbers, etc. In addition, the blue print could be designed by an expert architect or by an inexperienced house designer. Furthermore, the builders might be skilled and experienced or very inexperienced. The end result of these possible interactions will determine the quality of the new house. The same analogy can apply to the outcome of a conception that will lead to a human being after the interaction of the inherited genes with the unique environmental factors.

Mutations can be of many types, namely, a specific change in a single base in the DNA of a single gene. It could also be a duplication or deletion of bases in that gene. Moreover, gene amplification or deletion of a normal gene is considered a mutation. Further, alterations in the numbers (deletion or amplification) of whole or parts of a chromosome are considered mutations. Finally, rearrangements of chromosomes are considered chromosomal mutations. Probably the best illustration of this concept of genes and environment (or "nature and nurture") is the human sun cancer – prone syndrome, xeroderma pigmentosum [7-9]. These individuals inherit a mutated gene from both parents that codes for a protein that is unable to repair ultraviolet light induced DNA lesions in skin cells. As a result, if those lesions are not repaired, the cell will either die if the lesions were too prevalent or if it survived via an "error of DNA repair" [10-12], it could have a mutated gene that could contribute to the "initiation" of a skin stem cell , which after clonal stimulation by a "epigenetic agent" (endogenous factor such as a growth factors, hormone, or cytokine) or by an exogenous agent (environmental pollutant, drug, dietary factor, etc.) that act as a "promoter", a skin cancer could appear. On the other hand, if after conception and birth, this xeroderma pigmentosum individual, if detected in utero, could be sequestered from sun light or the environmental trigger, and would not suffer the consequence of mutations by "errors of DNA repair" and have low risk to skin cancer [9]. Even though these XP individuals lack DNA repair of UV-induced DNA damage in internal organs, they could still be a low risk to non-skin cancers (UV light can only penetrate a few cell layers of the skin) because there is another source of mutations.

The example of Bloom's syndrome might serve to illustrate the second cause of gene mutations [13], namely via "errors of DNA replication" [14]. In this syndrome, which has normal DNA repair, gene mutations can occur via these "errors of DNA replication" every time a stem cell is forced to divide during normal tissue growth or wound repair [15]. This is probably the explanation of why lung cancers can arise in individuals that never smoke, or been exposed to down-stream smoke [16,17]. All of us must have various mutated stem cells in all of our organs, some of which could lead to cancer or stem-cell- derived diseases.

Today, we know that there exist mechanisms that can modify the expression of the inherited genetic information, namely, "epigenetic" mechanisms. The term, "epigenetic", means that one can modify the expression of the inherited DNA at the (a) transcriptional level (such as, methylation of DNA or histone proteins); (b) translational level , such as splicing of the original RNA message and micro-RNA modulation of the translational process; or (c) posttranslational modification of the finished protein by phosphorylation . In each of these cases, the original inherited DNA sequence has not been changed, but the ultimate coded information has been over- or under-expressed; modified during the translational process, or modified structurally or functionally at the posttranslational level. Clearly, each of these molecular/biochemical steps (transcription, translation, and post-translation) are distinct mechanisms. One must recognize that the transcription process is a "down-stream" process that occurs after the organism, organ, and cell receives a signal from the broad "environment" that triggers various intra-cellular pathways to "turn on" or "turn off" transcription, translation or post translation mechanisms. One of the early upstream events occurs at the cell-cell communication level.

The major hypothesis of this is that, while mutations do play a role in many diseases, both inherited genetic predispositions and somatic diseases, such as cancer, "epigenetic" mechanisms are the real drivers of most chronic diseases. Because the expression of normal genes after conception must take place in a very delicate, orchestrated manner during embryonic, fetal and neonatal development (since these genes will have only one chance to function properly), there is no going back after neonatal development to re-express these genes to undue any dysfunction during development. These "epigenetic" factors can be both endogenous (coded by genes that control hormones, growth factors or cytokines, etc.) or a wide range of exogenous (physical agents; pollutants, dietary factors, medications, behavior choices, such as smoking, alcohol, lack of exercise, etc.). To put the concept of "epigenetic" mechanisms in perspective, one must realize that "epigenetic" mechanisms are needed for normal development and maintenance of homeostasis during life. If those "epigenetic" mechanisms are made dysfunctional during critical periods during development or for sustained periods of time later in life, that could lead to one of five distinct cellular consequences of gene expression, namely, cell proliferation, cell differentiation, apoptosis, adaptive responses of differentiated cells and senescence. Then an epigenetic" alteration could lead to too many or too few cells proliferating (especially organ-specific stem cells), premature or postponed differentiation of stem cells; too few or too many cells dying by apoptosis; blockage of adaptive responses of terminally differentiated cells (islet cells in the pancreas or neurons in the brain), and to premature senescence of cells.

To illustrate these complexities of mutational and epigenetic interactions, there is another human genetic syndrome which will illustrate a complex interaction of chromosome mutations and epigenetic alterations that can lead to many human chronic disease states. The Down syndrome is one that can inherit an extra chromosome 21 or parts of chromosome 21. In other words, these individuals have been characterized as being born with "birth defects", predisposition to diabetes, cardiovascular diseases, leukemia, autism-like spectrum disorders, premature aging and if they live long enough, can manifest a high risk to Alzheimer's disease [18-20]. In other words, these chronic diseases do not have their origin in point mutations on the three copies of genomic DNA alleles, but have their origin in the abnormal gene regulation when three copies are expressed during development. In brief, these chronic disease consequences are primarily the result of epigenetic mechanisms caused by a chromosomal mutation.

So while many "genetically-predisposed" individuals who manifest their disease early in life are found in pediatric patients, there are some in whom the disease manifest themselves somewhat later in life. Many human geneticists estimate that these inherited gene mutations contribute to a relatedly small fraction of our diseases.

Therefore, the bulk of the non-hereditary diseases can be considered "Environmental".

Lastly, while the death of cells can be the direct cause of death of the individual or indirect contributor to acute and chronic diseases, based on when the cell death occurred, the type of cell that died, how much cell death occurred, in which organ the death has occurred, a few examples will serve to illustrate the contribution of cell death. Cell death can be caused by non-repaired excess DNA damage; non-specific cell damage; and by epigenetically altered gene expression leading to apoptosis. Obviously, cell necrotic death early in embryonic development can lead to embryonic lethality. The case of xeroderma pigmentosum skin cancer exposed to normal UV light or of the non-xp individual exposed to repeated high doses of UV light, leading to sun burns, can be at risk to skin cancer. Many of the exposed cells will die, with any mutated skin stem cells, caused by "errors of DNA repair", that survived, they could be forced to go into compensatory hyperplasia or wound repair. So, also, in individuals who drink too much alcohol (a non-mutagenic cytotoxicant) will kill hepatocytes and force the surviving liver cells, especially the liver stem cells, to replenish the liver by a compensatory hyperplasia. In these cases, cell necrotic cell death can be viewed as an indirect "tumor promoter" [21-24].

In these four cases, the XP, Blooms, Downs syndromes, plus the example of excessive cell death by either mutagenic or cytotoxic agents, can contribute to multi-factorial mechanisms of both acute diseases (acute food poisoning) or chronic diseases (atherosclerosis, cancer).

Environmental Medicine: How Chemicals, by Inducing Oxidative Stress, Trigger Signal Transduction to Alter Cell-Cell Communication and Gene Expression

Those physicians, who must deal with "Environmental Medicine", such as occupational physicians or those involved in environmental exposures, such as the exposed population of the poly brominated or polychlorinated biphenyls in Michigan or to mercury exposure in Minamata, Japan, have a very difficult task of knowing exposure levels, genetic background of those exposure, stage of development, gender, exposures to potential additive, antagonistic or synergistic factors, dietary or nutritional status of those exposed. To have experimental animal data to the presumptive environmental factor, thought to be a potential causative agent of a human disease, itself, does not necessarily provide additive information for predicting risk or determining the "cause" of any disease.

With the concept that human diseases should be viewed within the concept of "Nature and Nurture", rather than "Nature versus Nurture", and that "precision medicine" and "mutations as drivers" concepts, the traditional view of "Environmental Medicine" must move away from the idea that any exposure to toxic agents, such as radiation, pesticides, chemical pollutants, drugs, viruses, bacteria, fungi, heavy metals, endocrine disruptors , bad diets, nutrition, or abnormal behavioral, psychological, social and cultural factors, will ,alone, provide the needed explanation to prevent any disease associated with these exposures. This is not to suggest that these factors could not or did not contribute to these diseases, but that, by understanding the mechanisms by which these environmental factors work in the pathogenesis of these diseases, will it give more realistic approaches to prevention.

With the fact that radiation exposure to UV light can contribute to point mutations [10-12], and ionizing radiation can contribute to chromosomal mutations and cell death at high doses [25], the exposures to environmental chemicals (physical, dietary, behavioral cigarette smoking, medications, performance-enhancing drugs, etc.) will be assumed to work via "epigenetic mechanisms". This concept, while it challenges much of "environmental toxicological" paradigms [26,27], has assumed that these chemical toxicants were mutagenic when tested either in bacterial systems [28], or in *in-vitro* (abnormal or cancer cells) or *in-vivo* assays using the non-target cells (differentiated cells and not adult stem cells or using total genomic and mitochondrial DNA rather than pure genomic or pure mitochondria DNA) [24].

From this point on, it will be assumed that toxic chemicals, which can induce oxidative stress and free radicals in differentiated cells, which have many mitochondria, are "epigenetic" toxicants, not mutagens. This is a critical assumption, since most toxicologists believe that, once a cell encounters a chemical and oxidative stress, as well as the detection of a variety of free radicals and electrophilic chemicals are detected, the chemical has to be classified as a mutagen. Chemicals, such as phorbol esters, DDT, phenobarbital, phthalates, chloroform, alcohol, etc., while generating free radicals and oxidative stress, are not genomic mutagens. While it is correct that these free radicals can interact with the cellular macromolecules, such as proteins, nucleic acids, several important factors must be considered. First, there are chemicals that can be metabolized to form various electrophiles and other chemicals that are not metabolized. In addition, some cells can eject the incoming chemical because they express drug transporter genes, such as stem cells and cancer stem cells. Stem cells, unlike their differentiated daughters, do not have many mitochondria. Differentiated cells, which have many mitochondria and do not express drug transporter genes, can metabolize toxicants to form free radicals that can damage and mutate mitochondrial DNA. However, at normal concentrates no genomic damage or mutations occur. To mutate a few mitochondria in a cell would not affect the cells ability to survive. At toxic concentrations, mitochondrial damage could affect the cells survival and usually these sets off the apoptotic response. The oxidative stress can induced mitochondrial DNA damage, as well as induce intracellular signaling to alter gene expression [29]. Depending on the dose or concentration of these agents and the cell type, the biological consequence can be very different. To make the case that environmental factors work primarily as "epigenetic agents", let us examine chemicals such as cigarette smoke or grilled meat, (e.g., polyaromatic hydrocarbons, etc), TCDD, polybrominated - and polychlorinated biphenyls, DDT, asbestos, bisphenol A, arsenic, lead, etc. These chemicals are not genomic mutagens in human adult stem cells. These agents, while inducing oxidative stress at non-cytotoxic concentrations, can induce mitochondrial DNA lesions in differentiated cells. Stem cells have few mitochondria (the reason they metabolize glucose via glycolysis) and express drug transporter genes, such as ABCG2 [30-36].

There is probably an evolutionary reason stem cells in metazoans were resistant to toxicants, because if they exhibited the same sensitivity to these toxicants, the organ and organism would die

since they would not be able to replenish the dead cells. Stem cells, including "cancer stem cells" seem to be resistant to both radiation and environmental chemicals (chemotherapeutic drugs) [37].

To put some scientific mechanistic information to this idea that these "environmental toxicants" work, not by mutagenic mechanisms, but by epigenetic mechanisms when there is no apparent necrotic cell death associated with the exposure levels, the concept of "epigenetic toxicity" was born in the study of carcinogenesis. While the concept of "epigenesis" had its origin in Conrad Waddington's original hypothesis [38], only when the carcinogenic process was conceived as involving multi-stages and multi-mechanisms [39-40], did the emergence of a non-mutagenic component to this disease (and other diseases) come about with the demonstration that a component from croton oil or chimney soot could "promote, but not "initiate" skin cancer [41,42].

The multi-stage, multi-mechanism process was operationally described as involving, first, the irreversible alteration of a single cells' genome ("the initiation" phase.) This single "initiated" cell, while not a cancer", no longer could terminally differentiate but seemed to have been induced to become "immortalized". Subsequently, if that single initiated "immortalized" cell was resistant to cell death by apoptosis but could stimulated to divide by mitogenic or "epigenetic" agents (growth factors; cytokines, hormones; or exogenous environmental agents, such as DDT, TCDD, Asbestos, etc.) they could be promoted. However, it is critical to note that these epigenetic agents had "threshold" levels to work; they need to be given in a regular fashion for long periods of time; and they must be given in the absence of "anti-promoters", usually "anti-oxidants". These promoters were not genomic DNA damaging or mutagen agents, while they could induce oxidative stress and modulate gene expression [29].

While these early initiation and promotion studies could not determine the underlying mechanisms of "initiation" or "promotion", the fact that "initiation" of a single cell implied a mutagenic mechanism because the event was irreversible. However, it was the hypothesis that "promotion" was due to the inhibition of DNA repair that would lead to mutations [43] that stimulated the experiment that showed that the classic skin tumor promoter, phorbol ester, did not inhibit DNA repair or cause mutations in cells exposed to UV light. Rather, it was shown that phorbol esters, while inducing oxidative stress, reversibly inhibited Gap Junctional Intercellular Communication (GJIC) [44]. Later, many non-mutagenic tumor promoters were shown to reversibly inhibit GJIC [45,46].

To put gap junctional intercellular communication in the concept of Epigenetic toxicology and in the concept of Environment Medicine, one needs to know that, as metazoans or a multicellular organism, we have 20 connexin or gap junction genes [47] that help to determine over 200 differentiated cell types of our body. They exist in all our organs and are expressed uniquely a few at a time in our neurons, blood cells, liver cells, heart cells, etc.

These gap junctions can be modulated (increased or decreased) in function at the transcriptional, translational or posttranslational levels) by the endogenous or exogenous chemicals. Hormones [48], growth factors [49], cytokines [50], as well as DDT, phenobarbital, arsenic, lead, etc., can inhibit GJIC [45], while anti-cancer agents, such as green tea components, genistein and others [51], can either prevent the inhibition of GJIC by these epigenetic tumor promoters or by the induction of connexin expression [51].

Another very important observation must be kept in mind concerning exposures to these epigenetic, GJIC-modulating chemicals. Dose makes the difference. Retinoids have been shown to modulate GJIC [52]. At physiological levels, it can be an anti-promoter [53]. At pharmacological concentrations, it can be a tumor promoter [54]. To make the concept of epigenetic agents even more complex to the practical application to Clinical Environmental Medicine is the fact that, any given chemical can, under different conditions, have both positive and negative health consequences [29].

The example of thalidomide should serve as the example. Obviously, the human tragedy of pregnant women who took this drug, viewed at the time as an efficacious sedative, caused significant birth defects of the limbs if taken at a critical period in the development of the fetus [55]. In addition, it is an efficacious medication for the treatment of leprosy [56]. Today, it is being used in clinical trials to treat cancers [57]. It is well known to be a modulator of GJIC [58]. This fact has major implications, not only for FDA or environmental regulators, but also for those in Environmental Medicine as clinicians. The factors, such as when the individual was exposed, concentrations used, whether the individual was normal or diseased at the time of exposure, the organ being exposed, etc., are complicating factors that must be considered.

Exposure in Utero as the Modulator of Risk to Diseases Later in Life: The Barker Hyopothesis

While the usual concept of teratogenesis or birth defects, associated with exposures to toxic agents during development, as being the cause of cleft palate, spinal bifida, Downs syndrome, fetal alcohol syndrome, Minamata Disease, etc., a broader concept of diseases, induced by some mechanism (s) in utero has been conceptualized by many investigators, including Barker [59]. For example, exposure of pregnant women to Diethylstilbestrol (DES) [60] seemed to cause vaginal cancers later in life. Again, while early interpretations of the mechanism by which this chemical worked, mechanistically (i.e., via mutagenesis), it is now thought it works by epigenetic mechanisms. In other words, since GJIC is critical to control, homeostatically, cell proliferation, cell differentiation and apoptosis during development of the embryo and fetus, by inhibiting or inducing GJIC in specific organs, one could alter normal development.

However, another interpretation of how these "epigenetic chemicals" might affect early development to cause alterations of risk to diseases later in life. If the embryo or fetus is exposed to agents that might increase or decrease the numbers of organ-specific adult stem cells, the risk to any stem cell-associated disease would be affected, respectively [61,62].

The example of the study of the late health effects after exposure to atomic bomb radiation in Hiroshima and Nagasaki might serve as to illustrate this potential mechanism. First, the study of the health effects of atomic bomb radiation led to the observation that there was a risk of various cancers, such as leukemia, after young children were exposed or breast cancer decades later after young women were exposed when young [63]. The usual interpretation for the "cause" of these observations is that ionizing radiation caused point mutations to initiate the cancer process and post radiation exposures

to other factors promoted these initiated cells to become invasive and metastatic.

An alternative explanation has been offered. Based of experimental studies of the mutation properties of gamma and neutron radiation from the atomic bombs suggests that ionizing radiation is a rather poor point mutagen but a rather good chromosome mutagen at relatively high doses. This type of radiation in "initiation"/ "promotion" studies also suggests that this type of radiation is a poor "initiator" of carcinogenesis [64,65]. Unlike UV light, which is a very efficacious point mutagen and good initiator of skin cancer [66], there needs to be a re-think of how ionizing radiation might be related to the production of invasive, metastatic cancers, such as human breast cancer.

To begin this "re-think", we need to examine current concepts of the origin of carcinogenesis. There are two opposing hypotheses. The first is that the "target cell" for human cancer is the organ-specific adult stem cell or the "stem cell" hypothesis [67-71]. The other hypothesis is that a mortal, normal somatic differentiated cell can be "immortalized" or "re-programmed". In other words, the "de-differentiation" or "re-programmed" hypothesis explains the main mechanisms to start the "initiation" process. Today, based on the isolation of various human embryonic [73,74] and organ-specific adult stem cells [75], it should be possible to determine which of these two hypotheses might explain the origin of the carcinogenic "initiated" cell. Is it the adult organ-specific stem cell that has been blocked from terminal differentiation or is it any somatic differentiated cell that can be "re-programmed to become "embryonic like"?

Here, one must examine the definition of a stem cell as a cell that can either divide via symmetrical cell division to produce two daughters that maintain its ability to remain "stem-like" and to have extended or "immortal" life span, or to divide asymmetrically to produce one daughter to maintain stemness or "immortality", as its mother stem cell, and one that can produce a mortal, differentiated progenitor.

In the opposing hypothesis, a mortal differentiated cell has finite or limited cell proliferation ability, i.e., the Hayflick hypothesis [76]. Therefore, if these mortal cells can be "de-differentiated" or reprogrammed to become "immortal", then this might be compatible with what we believe we know about cancer cells. One characteristic of a tumor with is many cells is that it contains cells that can self -sustain the growth of the tumor. Today, we know all tumors and cell lines derived from these tumors contain "cancer stem cells" and "cancer non-stem cells".

With the Nobel-Prize winning experiment that has been interpreted as demonstrating a normal differentiated fibroblast cell could be "reprogrammed" to become an "immortalized", induced pluripotent stem cells ("iPS") after genetic modification with a few embryonic genes (Oct3/4, Sox2, Klf4, c-Myc). The definition of these "iPS" stem cells is that they must form teratomas when injected back into an adult animal. An alternative hypothesis has been offered to explain these results [71,77-79]. If, in the original experiment, which does produce "iPS" cells, the population of normal fibroblasts could contain a few adult fibroblast adult stem cells, which already expresses the Oct4 gene, addition of the exogenous embryonic Yamanaka genes would assist the survival of the few adult stem cells but have no selective effect on the differentiated fibroblast. After a number of passages, most of these transfected differentiated fibroblast will die ("go through crisis"), whereas the few transfected fibroblast adult stem cells will be selected. These would appear to be the "reprogrammed" "iPS" cells, when they are the original adult fibroblast stem cells. If "iPS" cells are formed during the initiation event of carcinogenesis, then there should be many teratomas formed in human adult cancers, whereas one finds carcinomas and sarcomas.

Experimentally, with the isolation of normal human adult breast stem cells [80], it was possible to test the stem cell and de-differentiation hypothesis. These adult stem cells remain "immortal" until they are induced to terminally differentiate. When induced to differentiate Oct4 is repressed transcriptionally and the connexin Cx43 gene is expressed [71]. These human adult stem cells normally express the Oct4 gene [71]. When these normal adult human stem cells are genetically modified with the SV40 gene, they not only remain immortal and are resistant to terminally differentiate, but the Oct4 gene still remains expressed. If after radiation exposure these SV40 immortalized human breast stem cells can produce a few cells that are tumorigenic, still expressing the Oct4 gene. If, after transfection with the Neu/ERB2 gene, a few of these weakly tumorigenic cells become highly tumorigenic, with the Oct4 gene still expressed [71]. On the other hand if the normal adult breast stem cell is induced to differentiate (repress expression of Oct4 and induce expression of the connexin43 gene) and treated the same manner of the normal human breast adult stem cell, no "immortalized" or "reprogrammed cells" were obtained that had Oct4 expressed and connexin 43 repressed.

Using Ocham Razor to explain these results, it seems the stem cell hypothesis, not the de-differentiation hypothesis, explains the origin of the "initiated" cell to start the carcinogenic process. Coming back to the atom bomb breast cancer story, one could re-interpret that results. With the Japanese population prior to the atomic bombs being exposed to the traditional low caloric, rice, tofu, raw fish, vegetable diet, the stature of the Japanese population was relatively small and the median life span of the Japanese women was one of the longest in the world. They had a very small background frequency of breast cancers. That is one reason the small attributable numbers of atomic bomb associated cancers was detected because the background frequency was so low. Had an atomic bomb been dropped in Detroit, Michigan at that time, it is problematic if one could have detected any breast cancers attributed to the radiation exposure because the background frequency was so high. Since caloric restriction is known to reduce the risk to various chronic diseases [81] and since it has been shown experimentally that natural ingredients in soy products (i.e., genistein and Bowman Birk inhibitor) can reduce the production of cancers [82,83], albeit under specific circumstances [84], the result of genistein and other inducers of differentiation on normal human breast stem cells suggests that the Japanese diet might have reduced the risk to breast cancers in the young atomic bomb exposed women [85,86]. The explanation might be that during early pregnancy of the Japanese women who ate the traditional Japanese diet caused the differentiation of the stem cells of both the breast and bone tissues in the developing female fetuses. After birth of these female babies, who were small in stature, they had few breast adult stem cells to react to the hormones at puberty. They women had small breast and few breast stem cells which might be target cells for breast cancers later in life. In addition, because these women lived longer, as their bones

deteriorated, they also had few bone adult stem cells to make new bones as they aged. Ergo, osteoporosis has been a major consequence of the stem cell modulation during development.

If this could be a mechanistic explanation for the Barker hypothesis, namely, to increase or decrease organ-specific adult stem cell during development, the recent attention to the apparent increase of autism-spectrum syndromes might be due to exposure, primarily, to epigenetic natural or synthetic agents, which might increase or decrease adult stem cells in various regions of the brain that control various neurological/behavior functions. While, of course, there could be some inherited gene mutations, which might contribute to these brain disorders, given the example of the numbers of epigenetic-acting chemicals that we get exposed to every day and given the example of the Downs syndrome (an epigenetic-syndrome, caused by a chromosome mutations that lead to abnormal gene dysregulation during in utero development) that can exhibit various autism spectrum patterns of behavior, it seems that studying potential agents that might influence brain adult stem cell numbers and agents that could block the these epigenetic agents, would provide a new direction of "Environmental Medicine".

Conclusion

Traditional views of environmental medicine has been directed, primarily, by epidemiological approaches and non-mechanistic animal experimental studies. To associate radiation, lead, PBB, arsenic, asbestos, etc. with known exposures have and can give some means to estimate probable "causes" of various diseases. However, without understanding the toxicological mechanisms by which any of these agents work, little hope for prevention, except for total elimination of exposures, to a suspected agent or treatment will be possible. The major problem with identifying epigenetic agents is the fact that, while the underlying biochemical signaling mechanism might be very different, the penultimate cellular mechanism seems to be shared, namely, the up -or down-regulation of cell to cell communication [62].

Therefore, modulating cell-cell communication during development could affect the homeostatic regulation of cell proliferation, differentiation or apoptosis, as well as the control of organ-specific adult stem cell numbers to affect risk, after birth to diseases later in life. Alteration of gene expression by these natural and synthetic epigenetic chemicals in adolescents, young and individuals, as well as the geriatrics persons, could affect stem cell-related diseases. The recent apparent rise in autism-spectrum disorders might be a classic example of an epigenetic-caused mechanism to explain the Barker hypothesis.

Lastly, for Clinicians involved in environmental/occupational medicine, understanding of the potential mechanisms of epigenetic toxicology should be incorporated into their education and practical training. In addition, research in developing newer clinical test to assist in the practical task of understanding and detecting epigenetic agents that could affect various diseases, as well as discovering newer approaches to counteract the epigenetic, organ-specific toxicities of these agents. In the revolutionary report [87], it has been a challenge to the environmental toxicity and environmental medicine field to come up with newer approaches because the traditional approaches have not served medicine that well. Here also, while the challenge to re-think Environmental Medicine paradigms will not be easy, it seems our science to date, forces us to go into another direction. Precision medicine, in principle, is forcing us to go in a new direction, but it has not incorporated the complexities of epigenetic mechanisms [88,89]. Even some new thinking in this area is being challenged [90].

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